RAP1-GTP, RAC1-GTP AND FMS-LIKE TYROSINE KINASE 3 LIGAND (FLT3-L) AS BIOMARKERS FOR EARLY DETECTION OF SEPSIS

RELATED APPLICATIONS AND GRANT SUPPORT

[0001] This application claims the benefit of priority of U.S. provisional application Ser. No. 62/660,635 filed 20 Apr. 2018 of similar title to the present invention, the entire contents of which are incorporated by reference herein.

[0002] This invention was made with government support under grant numbers R03AI092130, R21NS066429, 1P50GM085273 and R21 NS066435 awarded by the National Institutes of Health (NIH), MCB0956027, awarded by the National Science Foundation (NSF), OC110514, awarded by the Department of Defense (DOD) and NSF I-Corp 7775897 The government has certain rights in the invention.

FIELD OF THE INVENTION

[0003] The present invention is directed to the discovery that Rap1-GTP and FLT3-L and optionally Rac1-GTP can be used as biomarkers for the early detection of sepsis in patients exhibiting symptomology consistent with sepsis. In particular, the present invention is directed to methods, assays and kits which may be used to distinguish sepsis (infection) from systematic inflammatory response caused by sterile inflammation in trauma patients. The method may be used to diagnose bacteria infection and/or sepsis and monitor therapy of a patient to allow modification of treatment and/or cessation of treatment. Additional aspects of the invention relate to the use of these same biomarkers for the monitoring of therapy in the treatment of sepsis.

BACKGROUND AND OVERVIEW OF THE INVENTION

[0004] The clinical phenotype of sepsis is often indistinguishable from systematic inflammatory response, caused by sterile inflammation in trauma patients. This ambiguity may result in overtreatment with lifesaving standard therapies of antibiotics when a bacterial pathogen can not be ruled out on a short timeline. This is because bacterial detection generally relies on a blood test and does not always detect or accurately measure localized infection burden. In complex injury cases involving trauma, the clinical picture is often confounded by the question of whether the patient is infected or is instead displaying signs of "sterile inflammation". Given that therapeutic intervention is quite different in each instance, it is important to have a sensitive measure for distinguishing when a patient is or becomes infected so that antibiotics and anti-inflammatory drugs can be appropriately administered.

[0005] Sepsis affects patients from a broad demographic spectrum and across many disease states. Cases include the very young and elderly, the immunocompromised, those with cancer, those with trauma and other critically ill patients with co-morbid conditions. The estimated mortality rate of sepsis is 30% in the US.[1-4] Successful clinical intervention during sepsis depends on timely diagnosis that enables judicious administration of appropriate treatment regimens. Blood cultures remain the diagnostic gold standard tor identifying bloodstream infections, yet suffer from the long lag time and low sensitivity in obtaining a positive result. The diverse range of microbial agents that cause sepsis, local sites of injury and infection and patient heterogeneity further confounds accurate tracking of the pathogenesis of this disease.[5]-7 So far ~180 distinct potential biomarkers of sepsis are known.[8-12] The high number of targets has limited their prognostic value. Elevated procalcitonin and C-reactive protein have been used most widely, though have limitations in distinguishing sepsis from severe inflammatory disease, motivating continued search for biomarkers.[13] In an ongoing study, we have identified previously unreported early markers of bacterial infection associated with sepsis. In particular, we have identified Fms-like tyrosine kinase-3 ligand (Flt3-L) and activation of small GTPases such as Rap1, Rac1 and sometimes RhoA as early indicators of sepsis caused by bacterial infection. In addition, these markers are sensitive to the efficacy of antibiotic treatment and can thus be used as a clinical decision support tool for initiation or termination of antibiotic treatment.

[0006] It is well established that monocytes are central to the innate immune response to infections that cause sepsis. The host response to bacterial infections includes the release of inflammatory mediators that stimulate the differentiation of monocytes into dendritic cells and macrophages, which subsequently migrate from the blood into tissue infection sites.[14] At the infection sites dendritic cells are effective and versatile antigen-presenting cells for T cell activation and coupling to the adaptive immune response, while macrophages serve to clear damaged cells and bacteria.[15] At the more granular level, Flt3-L is a strong stimulator of monocyte differentiation into dendritic cells.[16, 17] Flt3-L in turn stimulates activation of small GTPases such as Rap1, Rac1 and RhoA, the first two being particularly important in early sepsis diagnosis. GTPase activation is essential for the motility of leukocytes, involved in early response to host infection. Rap1 is needed for integrin-mediated cell adhesion and is a critical factor in the regulation of T-Cell and antigen-presenting cell interactions. (reference: Katagiri K, Hattori M, Minato N, Kinashi T Rap1 Functions as a Key Regulator of T-Cell and Antigen-Presenting Cell Interactions and Modulates T-Cell Responses. *Mol. Cell Biol.* 2002; 22:1001-1015). Rac1 and RhoA are central to cytoskeletal remodeling required for leukocyte motility and extravasation through tissue to points of inflammation (references: Cherfils J and Zeghouf M. Regulation of small GTPases by GEFs, GAPs, and GDIs. *Physiol Rev.* 2013; 93: 269-309; Lemichez E and Aktories K. Hijacking of Rho GTPases during bacterial infection. *Exp Cell Res.* 2013; 319: 2329-36.)

[0007] To establish a possible mechanistic link connecting GTPase activity, bacteria virulence factors and innate immune response to bacteria, we measured GTPase activity in cell lysates challenged cells with lipopolysaccharide (LPS) a Gram-negative bacterial endotoxin, which stimulates GTP binding to Rap1 in phagocytes and endothelial cells via CD14[18] (FIGS. **1**A & B). In parallel, other cells were exposed to Flt-3L, which elicited GTP binding to Rap1 and Rac1 (FIGS. **1**A & C). Given the in vitro associations between Flt-3L and Rac1-GTP, we hypothesized that bacterial infection stimulates coordinate-increases in Flt-3L and Rap1-GTP. Furthermore, based on our data we hypothesized that LPS found in Gram negative bacteria, is likely to synergistically upregulate Rap1-GTP in tandem with Flt-3L which stimulates the upregulation of both Rap1-GTP and